

A Simple Device for Making Successive Photomicrographic Records of Large Groups of Developing Organisms¹

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IN OUR analysis of the effect of ionizing radiation upon the cleavage of sea urchin zygotes we found it necessary to take successive photomicrographs of a large number of eggs, in different samples, which had been exposed to graded doses of ionizing radiation (Hsiao and Daniel, 1960). In order to estimate the rate of cleavage of the irradiated samples of fertilized eggs it is highly desirable to follow the cleavage of each egg in every sample and make photomicrographic records for later analysis. In other words, we need to take time-lapse pictures of the developing eggs subjected to different amounts of radiation so as to calculate the rate of cleavage and correlate it with dosage. After some preliminary trials we have put together, using commonly available materials, a simple device capable of taking photomicrographs repeatedly from the same field in a series of samples of irradiated eggs at specified time intervals. By lining up, according to time, prints made from each field, each egg can be identified and its cleavage followed from the first to the last frame in the series, and its rate of cleavage can be calculated. It occurs to us that investigators who have occasion to record developmental and other recurrent phenomena may find this simple device useful. A brief description of its method of construction and manipulation is reported in this paper.

PRINCIPLES OF CONSTRUCTION

The simplest way to obtain a sequential record of a number of fertilized eggs after their exposure to irradiation or other treatment would

be to place the eggs on the stage of the photomicrographic instrument and take cinematographic or time-lapse pictures. This requires one set of instruments for each sample exposed to one specific dose. The cost of equipment and the limitation of laboratory space would rule out all sample series of reasonable size. However, if the samples are immobilized and the photomicrographic camera is brought over an exactly predetermined point in each sample to take time-lapse photomicrographs during the course of cleavage, it would be possible to make photographic records of a number of samples with one instrument. This can be done by: (1) Immobilizing samples of eggs contained in uniform-sized culture vessels, such as a culture cell on a 1- × 3-inch micro slide, in a straight line on a stage supported by a steady stand independent of the photomicrographic camera, so that when the microscope moves no motion is transferred to the samples. (2) Using a photomicrographic camera carriage made to move back and forth along a straight track placed parallel to the line of the immobilized sample cultures and adjusted to bring the microscope objective across the middle of the cultures. (3) Employing a series of uniform spacers (such as 1-inch rectangular bars for use with the 1- × 3-inch micro slide containing samples of egg cultures), whose width equals the distance between the centers of the culture vessels of two neighboring samples, to stop the photomicrographic camera carriage on the track each time the objective of the microscope reaches the center of a sample on the immovable stage. In this way a single photomicrographic camera can be used to photograph the eggs in the exact center of each sample culture vessel, and a series of the time-lapse photomicrographs is obtained for later analysis.

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MATERIAL USED AND METHOD OF CONSTRUCTION

The device built on the above principles consists of two independent parts: (A) a steady stand for supporting the cultures to be photographed, and (B) a movable carriage on which a microscope and its camera and substage lamp are mounted so that the microscope and its accessories can travel from culture to culture without disturbing the stand or the organisms on it. The materials used for the construction of this device are limited to the few simple items listed below:

- 7-ft length of 2×2 -inch wood
- piece of $28 \times 8 \times 1$ -inch plywood
- 12-ft length of $\frac{1}{2}$ -inch wide $\frac{1}{8}$ -inch thick angle aluminum
- $20 \times 8 \times \frac{1}{8}$ -inch sheet of aluminum
- 1 dozen No. 8, 1-inch wood screws
- 8 self-tapping sheet metal screws, size $3/32$ - $1/4$ -inch
- 3 aluminum sheaves, $5/8$ -inch in diameter
- 11 machine screws with nuts, size $3/32$ - $1/4$ -inch
- 6-ft length of 3-sided 1-inch-square extruded aluminum channel
- piece of $5\frac{1}{2} \times 28 \times \frac{1}{4}$ -inch plate glass

All these are easily obtainable from local hardware stores.

A. *The stand.* This independent part of the instrument is made of a rectangular wooden base, two aluminum trapezoidal end supports, and a rectangular glass toppiece or stage. The construction of these three parts is summarized below. (1) From the 2×2 -inch lumber, two 11- and two 31-inch lengths are cut. These four pieces are joined at right angles by means of either end-lap joint or pinned tenon-and-tusk joint into a rectangular base measuring 31×11 inches on the outside. (2) From the $\frac{1}{8}$ -inch-thick sheet of aluminum two 8×8 -inch pieces are cut and each is shaped into a near trapezoid (it will be referred to as trapezoid in this paper for simplicity), as shown in Figure 1A, by cutting off a triangular piece whose two sides adjacent to the right angle are 2 and 6 inches long (area ABC in Fig. 1A). After rounding the corners, two $5/32$ -inch holes

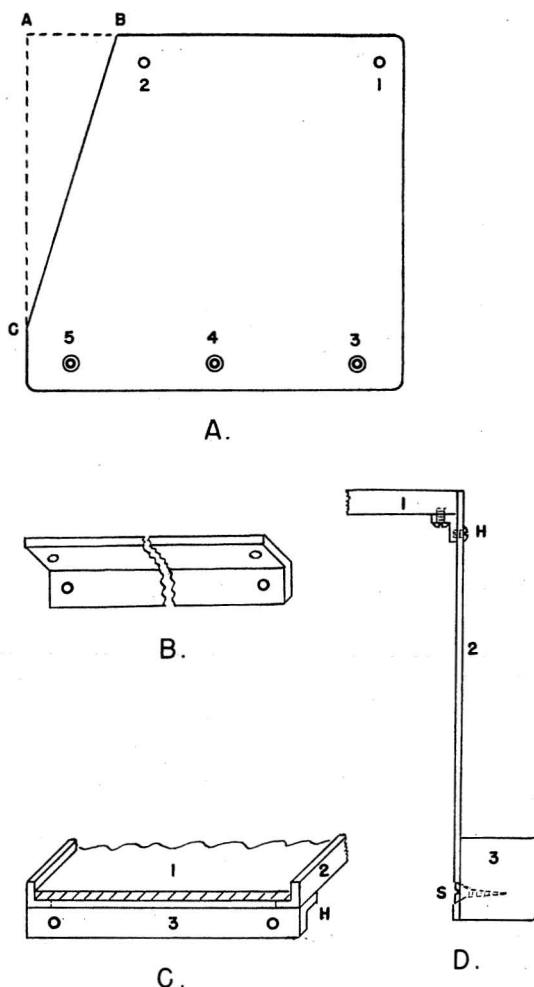


FIG. 1. Parts of the stand. A, Aluminum end support used as left end of stand. 1, 2, $5/32$ -inch holes; 1 being 1 inch from vertical edge and 2 5 inches from 1; 3, 4, 5, are three beveled holes 3 inches apart, 3 being 1 inch from vertical edge, and all $\frac{1}{2}$ inch from the bottom. B, Diagram of angle aluminum end frame for plate-glass toppiece or stage. Two upper holes $5/32$ inches, two lower ones $1/8$ inch. C, End portion of plate-glass toppiece or stage showing plate glass 1, mounted between two $28\frac{1}{2}$ -inch strips of angle aluminum, 2, which are attached on end piece, 3. The two $1/8$ -inch holes, H, are for mounting the toppiece or stage on the trapezoid end support. D, Sectional view of right end of stand, showing method of mounting trapezoidal end supports, 2, on wooden base, 3; and toppiece or stage, 1, on the trapezoid, 2. S, No. 8 wood screw; H, sheet metal self-tapping screw.

5 inches apart are drilled $\frac{1}{2}$ inch from the top and $\frac{1}{2}$ inch from the vertical edge of one of the trapezoids. Three $\frac{3}{16}$ -inch holes spaced 3 inches apart are next drilled $\frac{1}{2}$ inch from the bottom and 1 inch from the vertical edge. The holes on the second trapezoidal end support are similarly drilled but are done from the opposite surface so that this piece of aluminum forms a mirror image of the first one. (3) The rectangular glass toppiece or stage is made from a $5\frac{1}{2} \times 28$ -inch strip of $\frac{1}{4}$ -inch plate glass and a rectangular frame made of $\frac{1}{2}$ -inch wide angle aluminum. Two $5\frac{3}{4}$ -inch sections are cut from the aluminum stock and four holes drilled in each piece. Along one side of the aluminum angle two $\frac{5}{32}$ -inch holes are drilled each $\frac{3}{8}$ inch from the end, along the other side, two $\frac{1}{8}$ -inch holes, each $\frac{1}{2}$ inch from the end, are also drilled, as shown in Figure 1B. Two $28\frac{1}{2}$ -inch lengths of aluminum are cut from the same stock and two $\frac{1}{8}$ -inch holes are drilled on each piece, each hole being $\frac{3}{8}$ inch from the end. The 28-inch long plate glass is placed lengthwise between the inner surfaces of the long aluminum angle strips whose ends are then placed across the two short ($5\frac{3}{4}$ inches) end pieces in such a way that the outer surfaces of the four angle strips are in contact and the pre-drilled $\frac{1}{8}$ -inch holes on the long strips match the $\frac{5}{32}$ -inch holes on the short ones, as shown in Figure 1C, leaving the $\frac{1}{8}$ -inch holes of the short strips exposed at the ends of the frame. Four $\frac{3}{32}$ - $\frac{1}{4}$ -inch self-tapping sheet metal screws are driven through the $\frac{5}{32}$ -inch holes in the short end aluminum section tapping into the $\frac{1}{8}$ -inch holes in the long sections to hold the aluminum frame and glass together. It will be noticed that all the $\frac{5}{32}$ -inch holes are for the passage of, and the $\frac{1}{8}$ -inch holes for the tapping by, the self-tapping sheet metal screws.

To assemble the stand the two trapezoidal end supports are used to join the glass toppiece or stage to the wooden rectangular base. After placing the wooden base on a flat bench or table top, with its long sides running from left to right in front of the operator, the right trapezoidal end support is clamped with a C-clamp on the inside of the 11-inch-long end piece of the wooden base so that the vertical edge of the trapezoid faces the front of the stand, farthest from the operator and $\frac{1}{4}$ inch

from the right distal corner of the rectangular base, the slanting edge nearest the operator, the bottom of the trapezoid flush with the bench top and the smaller openings of the three beveled holes against the inside surface of the wooden end piece. After drilling three holes into the wood, using the holes in the aluminum trapezoid as guides, three No. 8, 1-inch, wood screws are driven into the wood until their heads are flush with the aluminum (see Fig. 1D at S).

The C-clamp is released. Similarly the left trapezoidal end support is screwed on the inside of the left end piece of the wooden base. The aluminum framed glass toppiece or stage is fastened to the trapezoidal end supports by apposing the two $\frac{1}{8}$ -inch holes on each end angle aluminum piece (H in Fig. 1C) to the two $\frac{5}{32}$ -inch holes near the top of each trapezoid and a round-head self-tapping sheet metal screw driven from the outside through the trapezoidal end support, tapping into the angle aluminum as shown at H in Figure 1D. When properly assembled this stand is very steady and can support a large number of culture slides or vessels on the plate glass.

B. *The carriage.* This part of the instrument consists of a platform with track, a cart for carrying the microscope and its accessories, and a number of spacers of uniform size. (1) The platform is made from a piece of $1 \times 28 \times 8$ -inch plywood and two strips of angle aluminum. Each piece of aluminum is cut 28 inches long and two $\frac{5}{32}$ -inch holes drilled through the apex of the angle, $\frac{1}{2}$ inch from each end, and beveled. One piece of the drilled aluminum is placed along the edge of the plywood board with the apex pointing upward and a No. 8, 1-inch, wood screw driven through each hole into the wood. The second piece of angle aluminum is placed parallel to the first, but with its apex $5\frac{7}{8}$ inches from that of the first, and fastened on the wood in a similar way. These two pieces of angle aluminum form the track on which the microscope-carrying cart moves. (2) The microscope-carrying cart is constructed from a piece of the $\frac{1}{8}$ -inch sheet aluminum, $5 \times 6\frac{1}{4}$ inches in size, with three pieces of angle aluminum and three $\frac{3}{8}$ -inch aluminum sheaves attached to its under surface. Six $\frac{5}{32}$ -inch holes, as indicated by No. 1-6 in

Figure 2A, are drilled through the sheet aluminum which forms the floor of the cart. A $\frac{1}{4} \times \frac{3}{4}$ -inch slit is cut at A and a round $\frac{1}{4}$ -inch hole at B, as shown in the same figure. Slit A is used to adjust and fix the substage illuminator in place, and B is for the microscope set screw, the same screw which came with the microscope for fastening it to the bottom of the microscope case during shipment. Two 5-inch sections of angle aluminum are cut, and two $\frac{5}{32}$ -inch holes drilled on each limb of the right angle. Each hole is $\frac{1}{2}$ inch from the end of the aluminum section. One of these two sections is mounted on the front undersurface of the floor of the cart with two $\frac{3}{32}$ machine screws and nuts, and the other section similarly mounted on the right undersurface at right angles to the first (Fig. 2B at 4). A third piece of angle aluminum, $1\frac{1}{2}$ -inch long is cut

and a $\frac{5}{32}$ -inch hole drilled at a distance of $\frac{1}{2}$ inch from each end. It is mounted along the back border on the undersurface of the cart floor with metal screws and nuts through openings 3 and 4 (see Figs. 2A, 2B). Three aluminum sheaves (1, 2, 3 in Fig. 2B) are mounted with machine screws and nuts on the vertical limb of the angle aluminum pieces on the undersurface of the cart floor so that sheaves No. 1 and 2 are $4\frac{1}{2}$ inches apart and each $\frac{1}{4}$ inch from the end of the 5-inch section of angle aluminum. Sheave No. 3 is mounted on the center of the vertical limbs of the $1\frac{1}{2}$ -inch section of angle aluminum and at a distance $5\frac{7}{8}$ inches from a line joining sheaves No. 1 and 2. When the cart is placed on the platform the sheaves should ride smoothly but snugly on the track. (3) The spacers are six $5\frac{3}{16}$ -inch sections cut from the 1-inch extruded rectangular aluminum channel. One spacer is mounted on the right-hand end of the platform with No. 8 wood screws, after the proper holes are drilled, to serve as the starting or ending point in the side to side movement of the microscope-carrying cart.

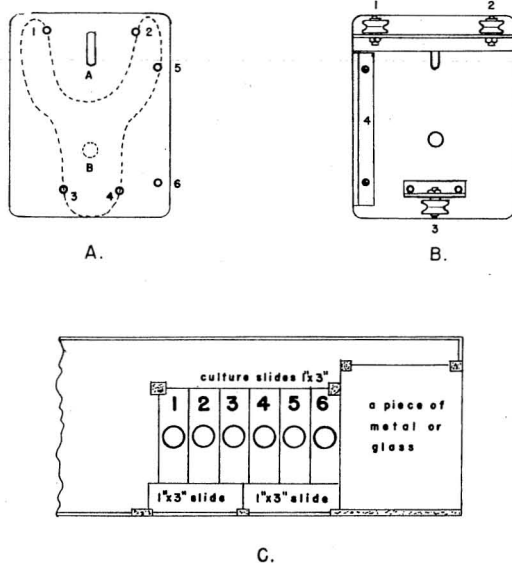


FIG. 2. Diagrams showing microscope carrying cart floor and top view of stand in use. A, Top surface of cart floor, showing openings 1 to 6 for attaching angle aluminum on undersurface; A, slit for adjusting and fixing illuminator; B, opening for microscope base set screw. Broken line indicates outline of the base of the microscope. B, Undersurface of cart floor. 1-3, sheaves; 4, 5-inch angle aluminum for buffering against spacer. C, Right portion of plate-glass toppiece of stand showing culture slides, 1-6 in place. Stippled areas represent masking tape. Other slides and glass (or metal) are positioning guides.

OPERATION OF INSTRUMENT

To employ this instrument for taking photomicrographs a microscope and a substage illuminator are mounted on the microscope-carrying cart. In our laboratory an old E. Leitz Wetzler microscope with its mirror removed is fastened on the cart floor by its set screw driven through opening B into the horse-shoe shaped base, and a Kohler illuminator through slit A. The light is centered and adjusted by standard procedures (Gray, 1958). The stand is placed on the laboratory table with its length parallel with the edge of the table and the slanting edges of the aluminum end supports near the operator. The platform is placed inside the stand so that a $\frac{1}{4}$ -inch clearing is produced on all four sides between the platform and the base of the stand. The microscope with its cart and illuminator is carefully put in place by sliding the microscope stage immediately under the plate-glass toppiece of the stand from back forward across the stand until the sheaves are over the track. The cart is lowered onto the track and pushed toward the right until it is

stopped by the fixed spacer at the end. A culture slide containing the sample is placed on the plate glass with its length perpendicular to the long axis of the glass toppiece and directly under the low power objective of the microscope. In our experiments a glass or aluminum ring $\frac{3}{4}$ -inch I. D. and $\frac{5}{32}$ -inch high is mounted in the center of a 1- \times 3-inch micro slide with epoxy cement to serve as a culture cell. When the culture slide has been centered, a piece of metal or glass is placed snugly against its right side and fixed on the plate glass with masking tape to serve as a slide end-stop. To facilitate lining up the other culture slides on the left side of this extreme right one the 1-inch wide space between the 28-inch-long aluminum frame on the side nearest the operator and the culture slide is filled with another 1 \times 3-inch slide at right angles to the end-stop (see Fig. 2C). Other culture slides are then easily placed in a straight line on the left of the original one as shown in Figure 2C by pushing them toward the right lower corner against the slide end-stop. After all the culture slides are placed in a straight line from right to left, the microscope-carrying cart is moved toward the left by drawing it along the track and the 1-inch-wide aluminum spacers are placed between the cart and the end piece on the platform, the number of spacers used being equal to the number of culture slides. As both the spacers and the culture slides are 1-inch wide the slide on the extreme left will be centered directly under the microscope. The culture slides and spacers are numbered correspondingly, both starting with No. 1 on the left and ending with the highest number on the right.

A photograph of the assembled device with the photomicrographic instrument in place is shown in Figure 3.

To take photomicrographs with this device, a camera is mounted on the microscope, and the specimens in the culture cell brought into view. Usually, before the first exposure is made, the specimens are centered and arranged on each slide, using a glass needle, so that a large number of individuals can be observed in each microscope field. Once the specimens are properly arranged, no other manipulation is needed, and they will stay in place throughout the course of the experiment. After the specimens

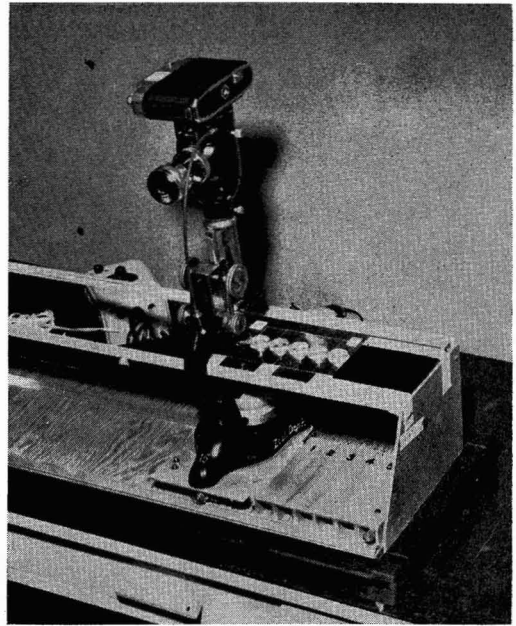


FIG. 3. Photograph of device, with camera, microscope, and illuminator in place.

are brought into sharp focus, a photograph is taken with the time of exposure and light intensity adjusted by the usual method for photographic work (Shillaber, 1944). To take a photograph of the second culture, the first spacer on the left is removed, the microscope pushed 1 inch toward the right tightly against the remaining spacers, the specimens brought into sharp focus, and a second frame of the film exposed. This procedure is repeated until the microscope-carrying cart is against the stopping spacer on the extreme right of the platform, and each culture has been photographed.

To start the second series of photomicrographs, the microscope is moved to the left, all the aluminum spacers replaced on the platform, maintaining their original order, and the cart pushed tightly against them. This automatically places the microscope objective over the center of the first culture. After a predetermined time lapse, the process of photomicrography described above is repeated, taking a second photograph of each one of the cultures, covering, in each case, exactly the same field as in the first run. The exact time of day a photograph is taken is noted down, and later

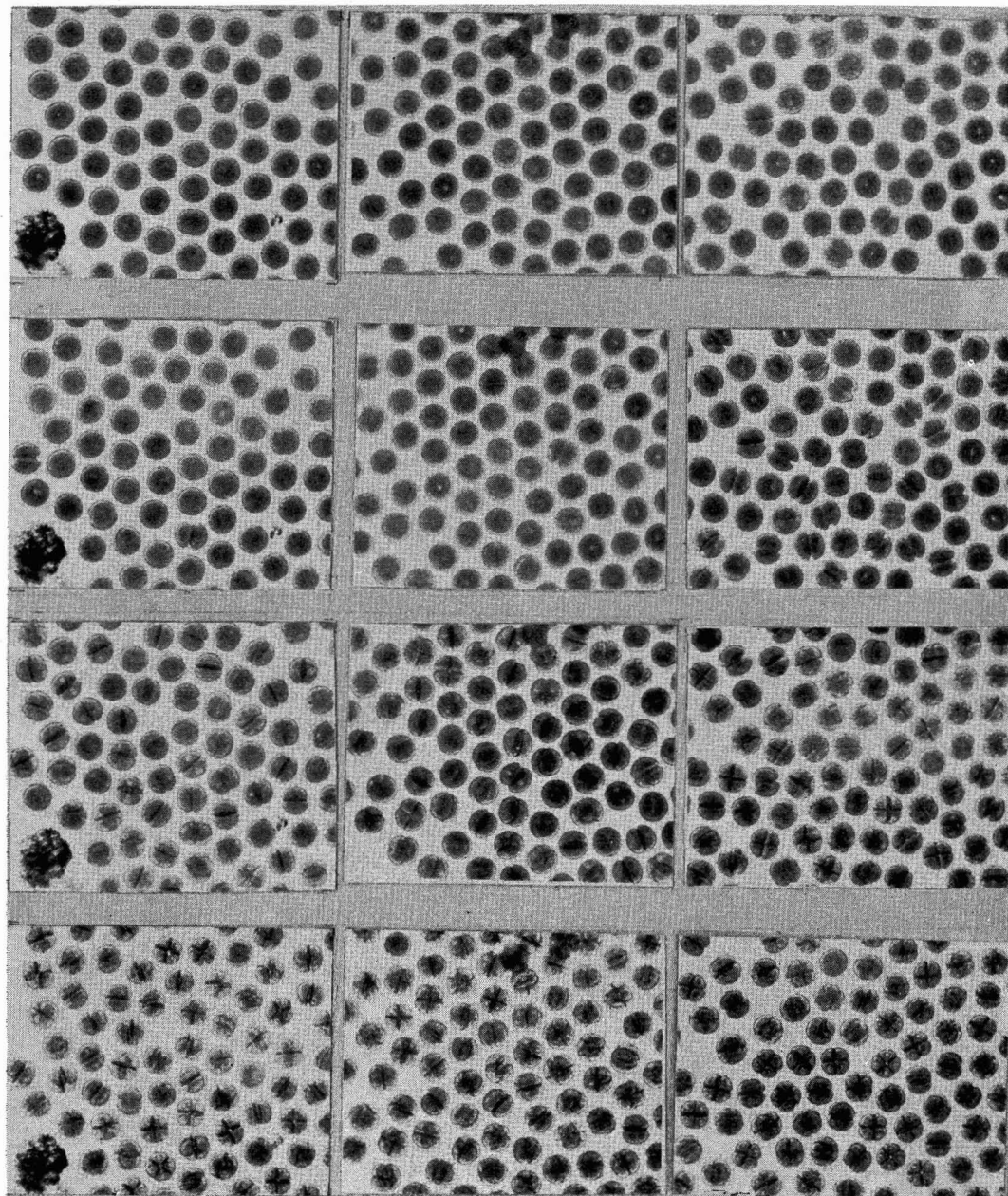


FIG. 4. Examples of time-lapse photomicrographs taken with the device. Each vertical column represents one sample culture in an X-irradiation experiment. Column 1 is the control, or Or; column 2 was subjected to 5r; and column 3 to 20r. The top horizontal row was taken at 1:25 P.M. or 85 min post-fertilization. The second horizontal row was taken at 1:30 P.M.; the third row at 2:15 P.M.; and the fourth row at 3:00 P.M. or 180 min post-fertilization.

transcribed onto the print. In one of our experiments, for instance, six cultures of eggs, each culture having been treated with a different dose of X-irradiation, were photographed successively within 3 min. In this way, a series of time-lapse photomicrographs, each bearing the time of day when it was taken, was made for each sample culture from the zygote to a desired stage, such as morula, and the resulting photographic prints analyzed and the rate of cleavage calculated. In Figure 4 selected examples of sequential photographs are reproduced to show the stability of the relative position of the eggs and their structural change during cleavage.

To identify each egg and estimate its rate of cleavage, a tracing of the first photograph of each sample is made on transparent paper. The drawing of each egg to be followed is given a number. By superimposing the tracing on the second or any subsequent time-lapse photograph of a particular sample culture, the eggs can be individually identified. The time it takes for an egg in a given sample culture subjected to a specific treatment to attain a certain developmental stage, (for instance, the attainment of the four-cell stage by a zygote after acute exposure to 20 roentgens), is determined by subtracting the time of fertilization from the time the picture was first taken which shows the desired stage of the egg. Thus, in one experiment the eggs were fertilized at 12:00 noon, the two-cell stage of egg No. 38 first appeared in the photograph taken at 1:30 P.M.; the four-cell stage at 2:15 P.M.; and the eight-cell stage at 3:15 P.M. Thus we can deduce that it took 90 min for 1st cleavage, 135 min for 2nd cleavage, and 195 min for 3rd cleavage.

DISCUSSION

1. Blum and Price (1950) described an apparatus for following photographically the cleavages of sea urchin eggs. They pointed out two advantages of the photographic method: (1) more accurate timing of the cleavages than fixing followed by counting; (2) the cleavages of each individual egg can be followed and recorded. Blum and Price used two microscopes in the inverted position, one for U-V-ray irra-

diated eggs, and the other for controls. The present device requires only one microscope.

2. In our instrument, observation is not limited to one treatment, i. e., one sample of treated animals compared with its control. In the present device, a large number of samples, each receiving a special treatment, can be compared with one or more untreated control or controls, using only one photomicrographic apparatus. A stand 28-inch long as used in our apparatus, can accommodate 20 or more samples at one time. The only limit to the number of samples is the time required for making one run of photography through the whole set of samples, and that required for making photographic print-analyses later. With this apparatus (as with Blum and Price's) the change in cleavage rate due to treatment such as X-irradiation can be recorded. For example an increase or decrease in the rate of cleavage can be observed; or initial retardation followed by recovery is made apparent. Similarly, the course of abnormal development can be followed; some of the abnormalities may disappear, others may persist. Fixing of eggs and later examination can not provide a sequential series of recorded events, especially recoveries. Furthermore, the eggs from a single female can be used in all of these samples, thus eliminating individual variations resulting from the use of batches of eggs from different females.

3. The instrument described here is one version of our device. Other modifications both in the material used and in details of construction are possible. For instance, we have also applied two coiled springs symmetrically to the microscope-carrying cart in order to pull the entire carriage tightly against the aluminum spacers. Another modification is the use of a light cooling and filtering device. A shallow transparent tray containing cold water can be placed on the plate glass between the culture samples and the light source.

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SUMMARY

A simple device for taking time-lapse photomicrographs of a group of cultures of eggs to show the development of each individual has been described. This device is made of readily available material. It consists of two parts: (1) A piece of plate glass supported by a frame which is steady and independent of the motion of the photomicrographic apparatus. It serves to replace the stage of the microscope and to carry the eggs in cultures in a motion-free state. (2) A carriage for the photomicrographic apparatus which can be made to travel back and forth in a straight line from left to right, repeatedly bringing the microscope and its camera to a specific field in each culture for sequential photomicrography.

The device has the advantage of being simple, easy to construct and operate, and it can be used to follow photographically an individual egg during its early development from zygote to morula as well as to record the appear-

ance, disappearance, or persistence of developmental abnormalities. Other recurring phenomena can also be studied photomicrographically by this instrument.

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